

PCR of

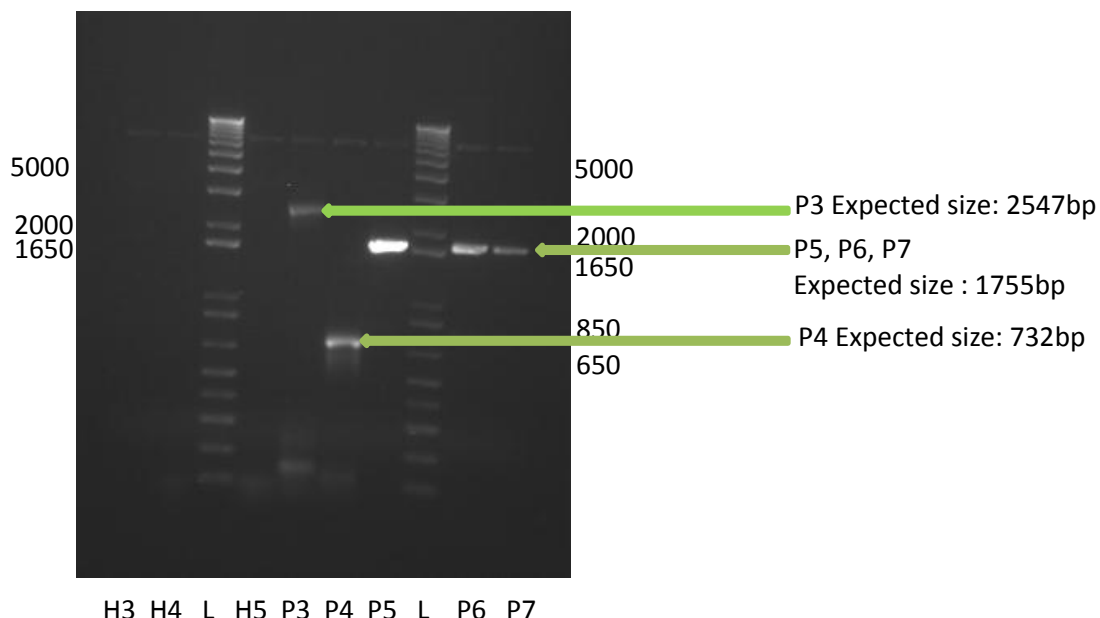
- sfGFP+hog1 flanking sequence H4
- split sfGFPC+hog1 flanking sequence H5
  - URA3+ rLuc overlap H3
- sfGFP+pbs2 flanking sequence P3
- split sfGFPN+pbs2 flanking sequence P4
  - Kan+ rLuc overlap P5
  - Kan+ nLuc overlap P6
  - Kan+ sfGFPN overlap P7

Reaction

Same for all

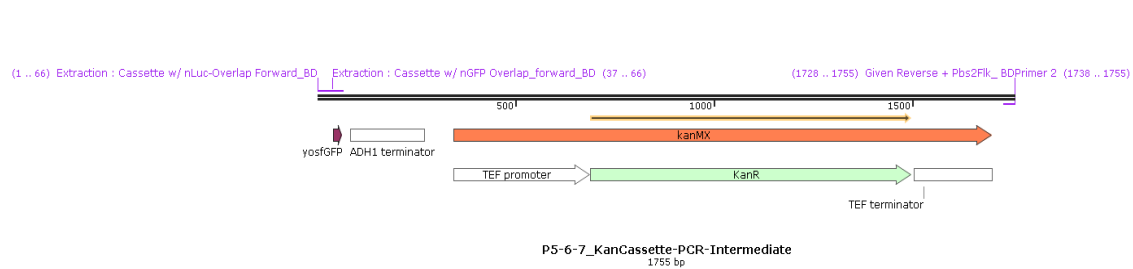
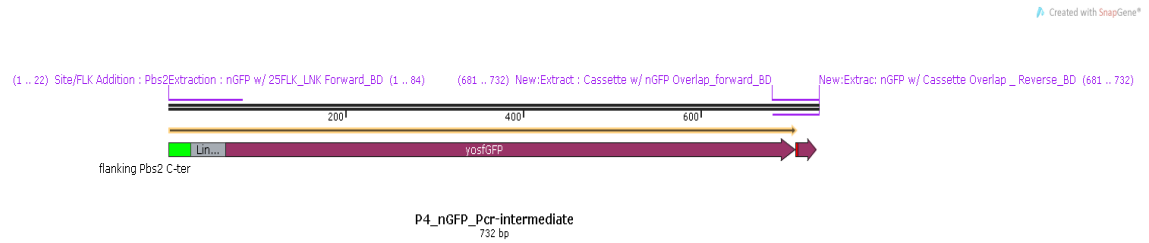
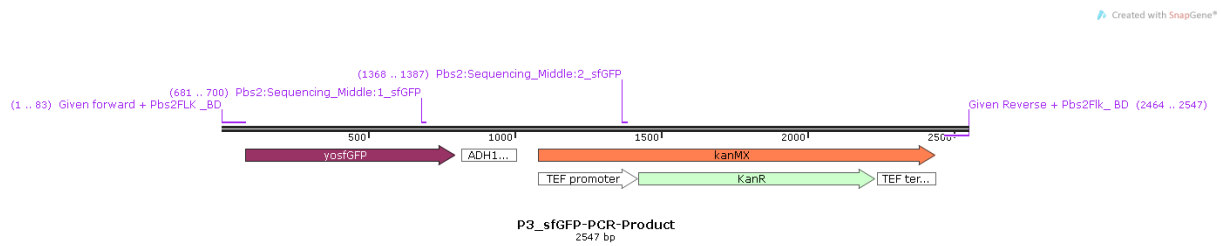
Template psfGFPURA3 or psfGFPKan	0.6
Forward primer 10mM	2,5
Reverse primer 10mM	2,5
Master Mix (with GC buffer)	11,5
Water	33

Results visualized by gel electrophoresis (1% agar, 120V, 40min)



For the H3, H4 and H5 we get nothin so we'll have to repeat the experiment.

Constructs we should have now:



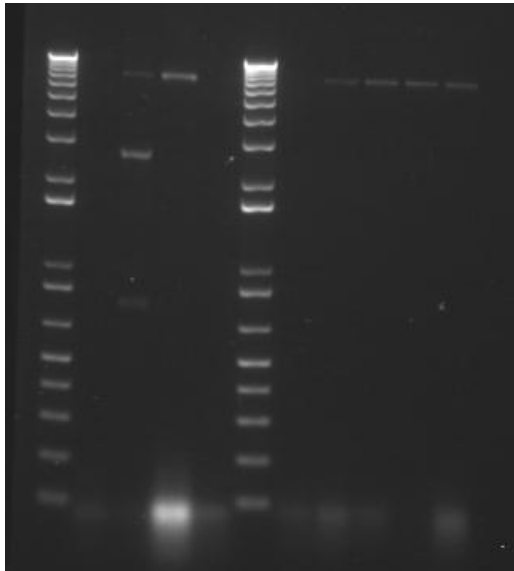
H3, H4, H5 second try: Gradient PCR  
Temperature gradient 58°C→66°C

25µL reaction (instead of 50µL)

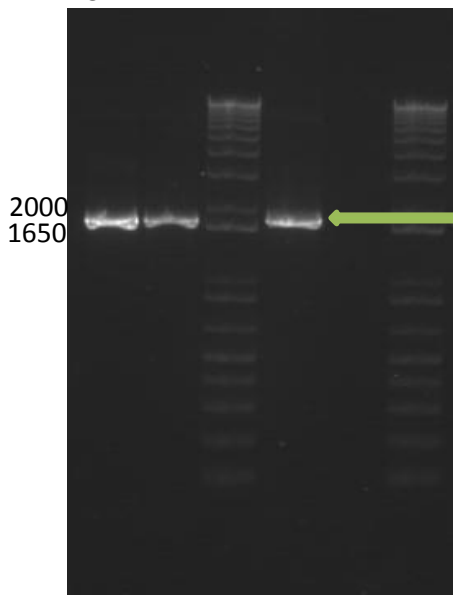
For all reactions:

Template psfGFPURA3	0,6
Forward primer 10mM	1,25
Reverse primer 10mM	1,25
Master Mix (with GC buffer)	5,75
DMSO	0,75
Water	15,4

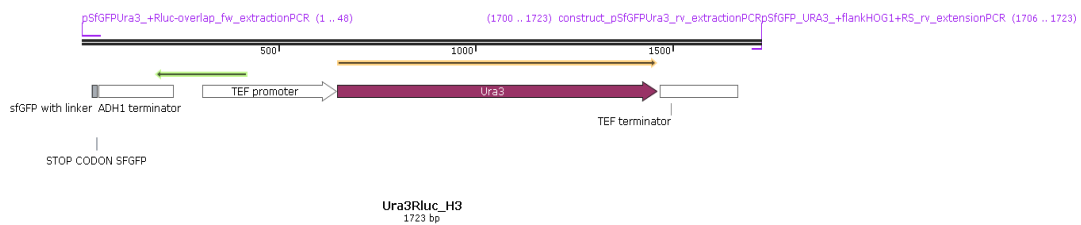
H4, H5: FAIL!!!



H3



H3 Expected size: 1723bp



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H3 samples concentrations was determined with Nanodrop

